--Through assays of normal cell function, we have identified dystroglycan as a laminin receptor signaling cytoskeletal and cell shape changes, and cell growth arrest in normal breast epithelial cells. Dystroglycan is a known transmembrane laminin receptor composed of two non-covalently linked portions, α-dystroglycan and β-dystroglycan; see U.S. Patent Number 5,449,616 hereby incorporated by reference. These originate from a single protein that is post-translationally cleaved. β-dystroglycan is embedded in the cell membrane. The extracellular chain, α-dystroglycan, binds to laminin. We have shown that inhibition of dystroglycan binding to laminin permits cell spreading and growth in the presence of laminin, conditions where cells would normally round-up and growth arrest. Results suggest a model whereby dystroglycan operates as a co-receptor, which organizes the laminin in the BM and facilitates signaling through other BM receptors. But, dystroglycan is shown to mediate shape changes and growth control without help from β1 and β4 integrins.--

A1

Please replace the paragraph beginning at page 17, with the following rewritten paragraph:

--Cleavage of α-dystroglycan was detected using cultured cells that cleave and shed dystroglycan from the cell surface. Dystroglycan cleavage was assayed for by immunoblotting to detect the presence of dystroglycan fragments in the medium of cultured cells. Mammary carcinoma cell lines SCg6 or TCL1 were cultured in 10 milliliters (ml) DMEM/F12 medium supplemented with 2% fetal calf serum, 5 μg/ml insulin (Sigma Chemical Co., St. Louis, MO), and 50 μg/ml Gentamycin (UCSF Cell Culture Facility). The cells were allowed to grow to 80% confluence in 10 centimeter (cm) plastic tissue culture dishes. The cells were rinsed two times with phosphate-buffered saline (PBS) and the medium was then changed to 10 mls DMEM/F12 medium supplemented with insulin, and Gentamycin, but without added serum. The cells were incubated in this serum-free medium for 48 hours, then this conditioned medium was harvested and filtered through 0.4 μm filters to remove cells or cell debris. The harvested medium was concentrated from 10 ml to approximately 1.0 ml using a centriprep-30 concentrator (Amicon, Beverly, MA). The concentrated supernatent was added to 1/10th volume of 10X sample buffer

A2

(50% glycerol, 100 mM acetic acid, 10% SDS (w/v), 12.5% (v/v) β-mercaptoethanol, bromophenol blue) and heated at 70 degrees Celsius for 15 minutes prior to loading on SDS-polyacrylamide (SDS-PAGE) gels, and subjected to immunoblot analysis. Assays of dystroglycan cleavage and shedding in the presence of the metaloproteinase inhibitor GM6001 (AMS Scientific, Pleasant Hills, CA) were performed in the same manner, with varying concentrations of GM6001, or the control C104 (AMS Scientific), diluted into the serum-free culture medium at the beginning of the 48 hour incubation. To compare treatments, equivalent volumes of conditioned medium from each treated cell population were loaded onto the gel to determine the relative quantities of dystroglycan shed into the medium.

Please replace the last paragraph of the specification that begins on page 24 and carries over to page 25 with the following:

--The tumorigenic cell line HMT-3522-T4 was found not to round-up in response to laminin when cultured on plastic, indicating that dystroglycan did not function well in these cells. In addition, this cell line is known to not to form organized acinar structures when cultured within a 3-dimensional gel of BM proteins (Matrigel), but instead displays the tumorigenic phenotype of disorganized and uncontrolled cell growth. Therefore, we over-expressed the human dystroglycan gene within these cells to see if, by restoring dystroglycan function, we could restore normal cell behavior to the tumorigenic T4 cells. Identical cells were also infected with an empty virus control (LXSN). We observed that the cells over-expressing the human dystroglycan gene respond to laminin in the medium by aggregating and rounding, whereas the control cells and rabbit dystroglycan expressing cells respond less. Placing these cells in the 3-D assay show that the T4 cells expressing the human dystroglycan gene no longer display the tumorigenic phenotype, but instead arrest growth and form organized acinar structures. Phase photographs of cultures showed the clear difference in colony size and organization; acinar-like structures were formed by cells over-expressing the dystroglycan cDNA, and disorganized structures are formed by the control population. α6 integrin staining showed the polarization of α6 integrins in dystroglycan over-expressing cells and the lack of polarity in the control

population. In addition to reverting the tumorigenic phenotype in culture assays, the cells possessing restored dystroglycan function did not produce tumors after subcutane us injecti n in to the flanks of nude mice (5 x 10^6 cells/injection), whereas the control cells did. These results reveal the role of dystroglycan as an important suppressor of tumorigenicity in cells. These results also demonstrate that restoration of dystroglycan to tumor cells is a novel therapeutic approach to slow or reverse the progression of cancer.

A3 cont

In the Claims:

Please cancel Claims 9-21 and 25-28.

REMARKS

By Office Action dated October 2, 2001, paper no. 4, Claims 1-8 and 22-24 were rejected. No Claim was allowed. Applicants affirm election made by telephone conversation with Examiner Owens, without traverse, of Groups I, II and V, Claims 1-8 and 22-24. Claims 9-21 and 25-28 are withdrawn from consideration.

Claims 1-8 and 22-24 are pending and under examination. Claims 1-8 and 22-24 were rejected. Non-elected claims 9-21 and 25-28 are cancelled without prejudice or disclaimer. Applicants reserve the right to file a Divisional application on the subject matter encompassed by those claims at a later date.

In conformity with current U.S. Patent and Trademark Office rules set forth in 37 C.F.R. § 1.121(c)(1)(i-ii), Applicants have attached hereto at Appendix 1, a separate sheet entitled "Version With Markings To Show Changes Made" to present the various changes made to the specification.

For the sake of clarity, the rejections and objections of the presently outstanding Office Action are set forth below, in the order in which they were presented and are herein addressed:

1. Claims 1-8 stand provisionally rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter not enabled by the specification.